

Prevalence of *Toxoplasma gondii* antibodies in red deer (*Cervus elaphus*) and other wild ruminants from Spain

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Abstract

Serum samples from 441 red deer (*Cervus elaphus*) and 161 other wild ruminant species, collected between 1993 and 2005 from six regions of Spain were tested for antibodies against *Toxoplasma gondii* by the modified agglutination test (MAT). Antibodies to *T. gondii* (MAT 1:25 or higher) were detected in 15.6% of red deer. Statistically significant differences were observed among sampling sites with seroprevalence in red deer from Catalonia (42.2%) being significantly higher compared with other Spanish regions (8.7%) ($P < 0.05$). Statistically significant differences were not observed between *T. gondii* seroprevalence and sex, age or management of hunting estates (open versus fenced). Seroprevalence of *T. gondii* infection in other ruminants species was 24% of 79 fallow deer (*Dama dama*), 21.8% of 33 in roe deer (*Capreolus capreolus*), 33.3% of three Spanish ibex (*Capra pyrenaica*), 20% of 10 chamois (*Rupicapra pyrenaica*), 10% of 10 barberry sheep (*Ammotragus lervia*), and 14.8% of 27 mouflon (*Ovis ammon*) in areas not including Catalonia, where no samples from these species were available. Serological results indicated a widespread exposure to *T. gondii* among wildlife in Spain and suggest that consumption of raw or inadequately cooked meat, as well as handling carcasses of wild game, should be taken into account as a source of infection for humans.

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1. Introduction

Toxoplasma gondii infection is widely prevalent in many species of warm-blooded animals, including humans (Dubey and Beattie, 1988). Wild and domestic felids are the definitive hosts, excreting oocysts in feces. Humans and a wide range of warm-blooded animals serve as intermediate hosts and can become infected by ingestion of food and water contaminated with sporulated *T. gondii* oocysts, by consumption of tissue cysts in infected animal tissues, or congenitally (Dubey and Beattie, 1988).

Public health concerns associated with *T. gondii* clearly indicate the need for epidemiological investigation of this infection in animals that can be used as a source of food. Cervids have been considered a source of *T. gondii* infection in humans worldwide (Dubey and Beattie, 1988). Some reports have shown that consumption of inadequately cooked or raw meat from infected cervids has caused acute and ocular toxoplasmosis in deer hunters in the United States (Sacks et al., 1983; Ross et al., 2001). Evisceration and handling of game may also represent risks for human infection (Dubey, 1994).

In the United States, white-tailed deer (*Odocoileus virginianus*) constitute one of the main reservoirs of *T. gondii* among wildlife (Hill and Dubey, 2002). Also, encysted *T. gondii* have been described in naturally infected mule deer (*Odocoileus hemionus*) (Dubey, 1982) and in experimentally infected elk (*Cervus elaphus canadensis*) (Dubey et al., 1980). In Europe, the main game deer species is red deer (*Cervus elaphus*). Viable *T. gondii* was isolated from naturally and experimentally infected red deer from New Zealand (Collins, 1981), and Scotland (Williamson and Williams, 1980). A few serological surveys of *T. gondii* prevalence have been performed in Norway (Kapperud, 1978; Vikoren et al., 2004) but, to our knowledge, no analysis of *T. gondii* seroprevalence has been reported in red deer from Spain.

Red deer, together with European wild boar (*Sus scrofa*), are the most popular and abundant big game species in Spain (Tellería and Saez-Royuela, 1986; Gortazar et al., 2000). Recently, we performed a study of *T. gondii* seroprevalence in wild boars in our country and observed a high rate, 38.4% of 507 wild boars analyzed (Gauss et al., 2005), indicating a widespread exposure to *T. gondii* in that species in Spain.

The aim of the present study was to provide information on epidemiology and prevalence of *T. gondii* infection in red deer from Spain. Other wild ruminant species such as fallow deer (*Dama dama*), roe deer (*Capreolus capreolus*), Spanish ibex (*Capra pyrenaica*), chamois (*Rupicapra pyrenaica*), barbary sheep (*Ammotragus lervia*), and mouflon (*Ovis ammon*) were also included in the study.

2. Materials and methods

2.1. Source of animals

Sera were collected from a total of 441 hunter-harvested red deer, 79 fallow deer, 33 roe deer, three Spanish ibex, 10 chamois, 27 mouflon and 10 barbary sheep shot during the hunting seasons from 1993 to 2005 in Spain. Fallow deer, roe deer, Spanish ibex, chamois, mouflon and barbary sheep samples were surveyed from similar areas as red deer with the exception of Catalanian area where samples from wild ruminant species other red deer were not available.

Red deer were classified into three age groups: young red deer (≤ 1 year old), juveniles (between 1–4 years old), and adults (≥ 4 years old). Management of hunting estates was classified as open (open hunting areas) or fenced (fenced hunting areas).

2.2. Sampling sites

Red deer samples were collected from three geographic regions of northern Spain: Principado de Asturias, Castilla y León (Burgos) and Catalonia (including the catalonian part of The Pyrenees); and from three geographic regions with five sampling sites of southern Spain (Andalucía (Cádiz and Sierra Morena), Castilla–La Mancha (Guadiana y Montes de Toledo) and southern Aragón (Montes Universales).

In the sampling sites from northern Spain, red deer live mainly in fenced estates (Burgos and seven of nine localities of Catalonia) with red deer living in open estates in Principado de Asturias and two localities of Catalonia (Vall d'Aran and Boumort). In the south, red deer and the other ruminant species sampled from Montes Universales live in open areas, while in other localities from Montes de Toledo or Sierra Morena,

there is diversity of management with the majority of animals living in fenced estates. In Cadiz and Guadiana all deer were sampled in fenced estates (a general sketch of the study sites can be found in [Vicente et al. \(2004\)](#)).

In the north of Spain, the Atlantic climate is represented by the region Asturias, a mountainous coastal region, with cool and damp weather for most of the year. The continental climate is dominant in Burgos, with cold winters and hot summers. Catalonia has a predominance of Mediterranean agro-ecosystems. In southern Spain, deer mainly live in the Mediterranean region, and the habitat is characterized by scrubland and evergreen oak (*Quercus ilex*) woodlands, with scattered pastures and small crops with less shade, less rain and a higher rate of evaporation than in the north. Guadiana, Montes de Toledo y Sierra Morena are characterized by high densities of wild boar and red deer due to intensive big game management.

Some of the sampling sites, including most of the areas of Catalonia located in The Pyrenees, Montes Universales, Montes Toledo and Sierra Morena and Asturias were mountainous areas.

2.3. Serological examination

Blood samples collected from the heart and/or thorax region of all game species during necropsy were centrifuged and sera obtained were stored at -20°C until assayed for antibodies to *T. gondii* by modified agglutination test (MAT) as described previously ([Dubey and Desmonts, 1987](#)).

Each serum sample was tested at dilutions of 1:25, 1:50, and 1:500. Positive and negative controls were included in each test. Sera with a titer of 1:25 or higher were considered positive and those with doubtful results were re-examined.

2.4. Statistical analysis

The statistical analysis was performed by chi-square test, using the SPSS 12.0 Statistical Program. The differences between variables were analyzed by Bonferroni or Tukey-Kramer tests. For multiple comparisons, Dunn's test was performed, and when variances were not homogenous, nonparametric tests were performed. The differences were considered statistically significant when $P \leq 0.05$. The confidence intervals (95% of confidence level) of prevalence were calculated based on [Martin et al. \(1987\)](#).

Seroprevalence was statistically analyzed considering the variables of sex, age, geographical areas (sampling sites) and management of hunting estates (open versus fenced).

3. Results

Antibodies (MAT 1:25 or higher) against *T. gondii* were detected in 69 (15.6%) of 441 red deer with titers of 1:25 in 39, 1:50 in 25, and $\geq 1:500$ in five. The prevalence of *T. gondii* antibodies in red deer in each sampling site is shown in [Table 1](#). Individual seroprevalence among areas varied from 0% to 44.2% and statistically significant differences were

Table 1
Prevalence of *T. gondii* antibodies in 441 red deer from Spain by sampling sites

Sampling sites	No. examined	No. positive ^a	Prevalence (%) \pm S.E. 95% C.I.
Northern Spain	118	40	33.9 \pm 8.5
Asturias	16	2	12.5 \pm 16.2
Burgos	16	0	0.0 \pm 0.0
Catalonia (and Pyrenees)	86	38	44.2 \pm 10.5
Southern Spain	323	29	9.0 \pm 3.1
Cádiz	7	0	0.0 \pm 0.0
Guadiana	100	11	11.0 \pm 6.2
M. Toledo	94	11	11.7 \pm 6.5
S. Morena	112	7	6.25 \pm 13.7
Montes Universales	10	0	0.0 \pm 0.0

^a MAT $\geq 1:25$.

observed in *T. gondii* prevalence among them ($P < 0.0001$). When grouped in northern and southern areas, antibodies to *T. gondii* were found in higher levels (33.9% of 118) in red deer from northern compared to southern Spain (9.0% of 323) ($p < 0.001$). However, these differences were related to the very high prevalence of *T. gondii* antibodies in red deer from Catalonia area (44.2%, $n = 86$, $P < 0.001$). According to ANOVA, statistically significant differences ($P < 0.001$) in seroprevalence of *T. gondii* were observed in red deer from Catalonia versus the other areas of the study: Asturias, Burgos, Guadiana, Cádiz, Sierra Morena, Montes Universales and Montes de Toledo. Deer from Catalonia had also the highest titers in the study, with five animals reaching titers 1:500.

No statistically significant difference was observed in seroprevalence in red deer when comparing hunting estate (open versus fenced) ($P = 0.113$). Antibodies to *T. gondii* were found in 20.9% of 86 red deer living in open areas, and in 14.0% of 349 deer living in fenced ones. There was no available data from six animals.

Antibodies were found in 13.1% of 199 of males and 16.9% of 236 of females, with no available data from six animals. No statistically significant difference of *T. gondii* seroprevalence due to sex was observed.

Statistically significant differences in antibodies prevalence of *T. gondii* and age were not observed. Antibodies were found in six out of 54 young, 10 out of 57 juveniles and 49 out of 321 adults red deer. There was no record from nine animals.

When data of *T. gondii* seroprevalence in red deer from Catalonia were examined in more detail, it ranged from 0% to 75% among nine different localities sampled (Table 2), although the differences were not statistically significant among localities. With the exception of Terrades locality, where seroprevalence was 0%, all the other localities sampled from Catalonia showed higher prevalence than any of the other areas in the study in both, northern and southern Spain. Prevalence levels in Catalonia were not related to sex, age or hunting estates. High seroprevalence levels were observed in mountainous areas of Catalonia (Table 2).

Statistically significant differences in seroprevalence of *T. gondii* in red deer in Catalonia were observed between years of sample collection ($P < 0.05$). Peaks of seroprevalence were observed in 1997 (75%), 1998 (83.3%) and 2002 (60%) with return to lower levels in 2003 (33.3%), 2004 (22.2%) and 2005 (22.2%), although always higher than mean values in other areas of Spain. There were significant differences between 2004 and 1997, and 2004 and 1998 ($P < 0.05$). However, it was observed that the years of highest prevalence were those when localities of highest prevalence were sampled (data not shown).

T. gondii antibodies were detected in 18 (24%) of 79 fallow deer, in seven (21.2%) of 33 roe deer, in one (33.33%) of three Spanish ibex, in two (20%) of 10 chamois, in one (10%) of 10 barbary sheep, and in four (14.8%) of 27 mouflon. No samples from these species were available from some areas, including Catalonia. No statistically significant difference of *T. gondii*

Table 2

Prevalence of *T. gondii* antibodies in 80^a red deer from Catalonia by sampling sites

Catalonia	Sampling sites	Hunting estates	No. examined	No. positive ^b	Prevalence (%) \pm S.E. 95% C.I.
Pyrenean–Pre-pyrenean mountainous areas	Boumort	Open	20	6	30.00 \pm 20.1
	Vall d'Aran	Open	12	6	50.00 \pm 28.3
	Andorra	Fenced	13	7	53.85 \pm 27.1
	Berga	Fenced	7	5	71.43 \pm 33.5
	Bellver	Fenced	10	5	50.00 \pm 31.0
Other areas	Vic (Osona)	Fenced	4	2	50.00 \pm 49.0
	Butsenit (Lleida)	Fenced	4	3	75.00 \pm 42.4
	Vallcalent (Lleida)	Fenced	4	2	50.00 \pm 49.0
	Terrades (Emporda)	Fenced	6	0	00.00 \pm 00.0

^a There was no available data from six animals concerning to sampling site.

^b MAT \geq 1:25.

Table 3

Prevalence of *T. gondii* antibodies in fallow deer, roe deer, mouflon, chamois, barbary sheep and Spanish ibex from Spain by sampling sites

Sampling sites	Species ^a								
	Fallow deer			Roe deer			Mouflon		
	No. examined	No. positive	Prevalence (%)	No. examined	No. positive	Prevalence (%)	No. examined	No. positive	Prevalence (%)
Asturias	40	7	18.2	18	4	22.2	–	–	–
Cádiz	–	–	–	7	2	28.6	–	–	–
Guadiana	11	1	9.1	–	–	–	11	1	9.1
M. Toledo	28	10	35.7	2	0	0	1	0	0
S. Morena	–	–	–	–	–	–	8	1	12.5
Total	79	18	22.8	33 ^b	7 ^b	21.2	27 ^c	4 ^c	14.8

^a Chamois: only sampled from Asturias. Barbary sheep: only sampled in Guadiana. Spanish ibex: only sampled in Avila.^b No data available of sampling site from six samples of roe deer (one of them *T. gondii* positive).^c No data of sampling site from seven samples of mouflon (two of them *T. gondii* positive).

seroprevalence in these species due to area was observed. Seroprevalence of *T. gondii* among different species could not be compared due to the different origin and relative small number of samples of other ruminant species (Table 3).

4. Discussion

The knowledge of the prevalence of different pathogens in wildlife species, including parasites, is of major importance for environmental protection strategies (Artois, 1993). The possibility of zoonotic diseases transmitted from wild animals to humans has important public health implications. The populations of red deer in Spain have grown over the last decades, due mainly to increased hunting management and human field abandonment. Red deer is one of the main wild ungulate species in Spain together with the wild boar and represent a potential source of zoonosis (Gortazar et al., 2005).

Reports of antibodies to *T. gondii* in cervids have been mainly reported in USA (Franti et al., 1975; Lindsay et al., 1991; Vanek et al., 1996; Ferreira et al., 1997; Dubey et al., 2004) but there are few serological data from *T. gondii* infection in red deer (*Cervus elaphus*) from Europe (Collins, 1981; Hejlíček et al., 1997; Kapperud, 1978; Vikoren et al., 2004), and to our knowledge none in Spain. Studies on prevalence of antibodies against *T. gondii* in red deer have shown similar rates in Czech Republic (15%, 46 out of 303) (Hejlíček et al., 1997) and in Norway (12%, 12 out of

99) (Kapperud, 1978) with the Sabin-Feldman dye test (DT). In our study we found 15.6% of seroprevalence of *T. gondii* in red deer in Spain by the MAT. Vikoren et al. (2004) observed lower seroprevalence in a more recent study of *T. gondii* infection in Norwegian red deer (7.7%, $n = 571$) using a similar agglutination test, although using a different cut-off titer.

In the present study, the 15.6% seroprevalence of *T. gondii* in red deer was not related to age, sex or hunting estates. The main factor affecting prevalence of infection was location. Seroprevalence to *T. gondii* in species on a given area depends on several factors such as presence of felids, climatic conditions, animal species examined and serological tests performed. Ours results showed significantly higher prevalence of infection (44.2%) in Catalonia, where red deer were sampled mainly from close to The Pyrenees, and suggested that higher exposure to oocysts in the catalonian environment was probably the main factor that contributed to the results observed. In other species, Cox et al. (1981) and Hejlíček et al. (1997) found considerable variation between localities and strongly positive sera came from animals collected in a small number of localized areas. Prevalence in areas with similar biotypes or soil types might be similar among animals from different areas (Smith and Frenkel, 1995). Areas with shade and relatively higher humidity have higher prevalence of infection, while areas characterized by less shade, less rain and higher rates of evaporation, and therefore more desiccation have lower prevalence rates (Smith and Frenkel, 1995). In the present study, similar lower prevalence of infection was

observed in different areas of Spain with mostly dry habitats while prevalence in the more humid mountainous areas of Catalonia was the highest.

Cats from urban Barcelona (Catalonia) had high prevalence of *T. gondii* antibodies (45% of 220), with the highest prevalence of infection in feral cats (Gauss et al., 2003); these seropositive cats probably already excreted oocysts and contaminated the environment in Catalonia. In Czech Republic, Hejlíček et al. (1997) observed higher prevalence in wild mammals from suburban areas where there was high density of domestic cats, and lower prevalence in areas extensively damaged by military activities where cats were less numerous.

Furthermore, the wild cat (*Felis silvestris*) population has been increasing in last years in Catalonia due to new governmental preservation programs to protect wild cats from extinction. At present, wild cats are widely distributed in Catalonia, mainly in mountain areas, where human settlements are scarce (Generalitat de Catalunya - Medi Ambient i Habitatge, 2005). Added to that, wild rabbits (*Oryctolagus cuniculus*) from humid forest areas of Catalonia showed the highest prevalence of *T. gondii* antibodies when compared with others areas from Spain (Almería et al., 2004). On the other hand, in a previous study in wild boars (Gauss et al., 2005) prevalence of *T. gondii* infection in Catalonia was low (two out of 27 samples). However, in that study, few samples were included. A more recent analysis of higher number of wild boar samples from similar areas as those described in the present study had significantly higher prevalence in Catalonia compared to the previous study (data not published). All these results taking into account seem to indicate Catalonia as one of the most important areas of *T. gondii* infection in our country.

Most of the sampled areas that had the highest prevalence of infection were located in or near The Pyrenees. Although low prevalence of antibodies has been observed in mountain regions in other studies (Franti et al., 1976; Kapperud, 1978), in the present study, both the highest seroprevalence and the highest antibody titers were found in red deer from mountainous areas of Catalonia. As mentioned above, wild cat populations are increasing especially in those areas. The high prevalence in Catalonia was most probably related to both large number of infected cats in the habitats and relatively higher humidity, conditions that

are more favorable for oocysts survival, spread and maintenance of *T. gondii* infection. More information concerning origin of red deer, translocations, presence of human settlements and cats will be necessary to identify which factors are influencing seroprevalence of *T. gondii* in red deer from Catalonia.

Statistically significant differences in seroprevalence of *T. gondii* in red deer were observed between years of sample collection in Catalonia. Our observations would be similar to those reported by Tizard et al. (1976) that have suggested that prevalence of *T. gondii* infection follow a cyclical pattern close related with climatic conditions. Tizard et al. (1976) have found that a 6-year cycle of high prevalence of toxoplasmosis occurred across Canada and suggested that decline in the percentage of seropositive was probably due to dry climatic conditions during the summer months that make survival of oocysts in environment not viable. Similar observations were reported by Williamson and Williams (1980) in Scotland. In the referred study, a peak antibody response was observed in 1974, and a return to low levels in 1975 and 1976. However, in the present study we have to take into account that the highest prevalence in Catalonia coincided in the time when localities of highest prevalence were sampled (data not shown). Therefore, further studies in Catalonia are necessary to provide more information concerning to these findings.

Other wild ruminant species were included in the present study, as fallow deer, roe deer, mouflon, barberry sheep and Spanish ibex. Unfortunately samples from other wild ruminant species other than red deer were not available in Catalonia. Due to the number of samples analyzed the present results should be considered as preliminary. We observed the highest seroprevalence in fallow deer. Few data of *T. gondii* infection are available from fallow deer. In Czech Republic, Hejlíček et al. (1997) found a very high prevalence of *T. gondii* antibodies in fallow deer (100%), although a low number of samples were analyzed (only three samples) and *T. gondii* was not isolated from the animals. Riemann et al. (1979) found 17% (11 out of 66) seroprevalence of *T. gondii* in fallow deer from areas of woodland forest, coastal shrub and open grassland of Central California Coast by the indirect hemoagglutination test.

In our study, higher total seroprevalence was observed in roe deer (21.2%) compared to red deer.

Kapperud (1978) observed antibodies in 63% of eight in roe deer (*Capreolus capreolus*) in lowland of coastal regions of Norway and Sweden. More recently, also in Norway, Vikoren et al. (2004) have observed that roe deer are more susceptible to *T. gondii* infection than red deer, and have suggested that, among cervids, roe deer is a potential source of infection for humans in that country. Similar results have been observed by Sroka (2001) in Poland (three out of three positive roe deer samples by MAT) in areas where a high rate of human toxoplasmosis was detected. On the other hand, Hejlíček et al. (1997) observed similar seroprevalence (14% of 95 roe deer) of *T. gondii* compared to red deer (15% of 303) in Czech Republic.

We observed similar seroprevalence of *T. gondii* infection in mouflon (14.8%) compared to the study of Hejlíček et al. (1997) in Czech Republic (10% of 20 mouflon samples). To our knowledge the present study shows the first report of seroprevalence of *T. gondii* in barbary sheep, chamois and Spanish ibex. Although a very high seroprevalence of *T. gondii* was observed among Spanish ibex (33.3%), very low number of samples was analyzed in our study.

With respect to environmental and public health implications, poor management and lack of adequate hygiene of hunters handling meat, organs and carcasses of wildlife, may play an ecological role in the transmission of *T. gondii* infection. Tissue cysts can survive for several days after the death of *T. gondii* infected animal (Dubey and Beattie, 1988) and hunters must be conscious of the importance of incinerate carcasses and residuals of hunting to avoid risk of infection to omnivores and carnivores – especially cats – because felids may contaminate the environment with oocysts which in turn may infect wild game that will later be consumed by humans.

In the present study, results suggest a widespread exposure of *T. gondii* among wildlife from Spain. The handle and consume of raw or undercooked infected wild game meat should be taken into account as a source of infection for human in our area.

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